Molecular effective quantification of pathogens and total flora in meat products

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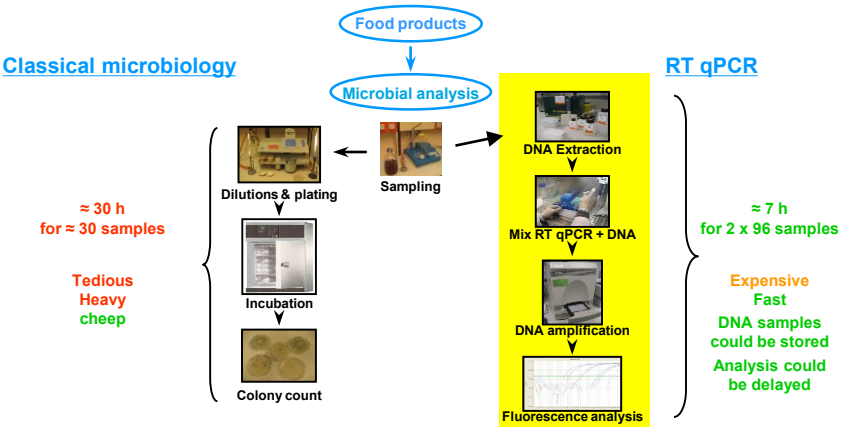
Introduction

Classical analysis for food shelf-life uses traditional microbiological techniques. Those are long, heavy and tedious. There is a demand from our industrial partners to develop a faster and easier alternative technique. Real time quantitative PCR (RT qPCR) has recently entered service in the field of food science and technology. Several methods have been developed for the detection and quantification of opportunistic pathogens and certain non-pathogenic spoilage microorganisms in food products. All molecular techniques needed an enrichment step which could hedge the quantification.

Objectives

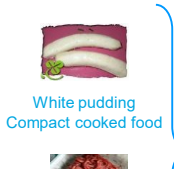
The aim of our study was to develop an effective molecular quantification for different flora of interest for food industry.



Challenges & Results

1 Extraction

➢ Efficient for all food matrices



- Lab extraction
- NucleoSpin Food
- NucleoSpin Tissue
- Qiagen Blood & Tissue

Tests with food matrices contaminated with pathogens or not

- Amount of microbial DNA
- Fluorescence analysis similar
- ➢ Quantification and purity analysis of extracted DNA
- ➢ Evaluation of the amount of microbial DNA

Comparison of technical & financial aspects

Faster
Cheaper
Effective on different food matrices

1 ml of sample
Centrifugation 10 min speed max
+ pure water
+ lysosyme
+ proteinase K
+ Tris-HCl 0,1 M pH 8
37 ° C
65 ° C
95 ° C
Centrifugation 5 min speed max
DNA in the supernatant

2 For pathogens

➢ Enrichment needed

➢ Possibility of quantification decreased or even impossible

TaqMan technology

	Listeria monocytogenes	*Salmonella* spp.	*Escherichia coli* 0157
Gene target	hly	ttrc	rfbE
Quantification threshold (cfu.cm^{-2} or .g^{-1})	13	6	11
Standard curve (standard deviation (%))	(± 5 %)	(± 6 %)	(± 6 %)

3 Total flora

➢ Composition varied according to tested food

➢ Original flora very diversified

SYBR Green technology

Gene target	16S rDNA
Quantification threshold (cfu.cm^{-2} or .g^{-1})	10^2
Standard curve (standard deviation (%))	(± 6 %)

Conclusions

For our industrial partners products, a microbial DNA extraction has been developed. This extraction could be applied on cooked and raw pork meat. Effective food pathogens and total flora quantification protocols has been performed. Standard curves have a low standard deviations, around ± 6 %. Quantification threshold for pathogens is around 10 cfu.cm^{-2} or .g^{-1}, and below, a detection is possible. The protocol of RT qPCR is a very interesting option for food industry, but more food matrices should be tested.

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